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PURINE EXCRETION IN FIVE SCORPIONS, A UROPYGID AND A CENTIPEDE

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Terrestrial arthropods differ in patterns of nitrogen excretion. Although insects generally are uricotelic, hypoxanthine and possibly xanthine have been identified in the moth, *Galleria mellonella* (Nation and Patton, 1961). Excreta of *G. mellonella* contained 2.6% uric acid and 0.2% hypoxanthine.

In contrast to insects, the principal nitrogenous waste of arachnids appears to be guanine. Only guanine was detected in the excreta of 34 species of spiders (Anderson, 1966). Anderson's work, in conjunction with the reports of earlier workers (Vajropala, 1935; Schmidt, Liss and Thannhauser, 1955; Atkinson and Chorlton, 1956; Haggag and Fouad, 1965), clearly illustrates the importance of guanine as the excretory product of spiders. Scorpions, however, are more inconsistent than spiders in their pattern of nitrogen excretion. Gregoire, Gregoire and Miranda (1955) reported high concentrations of guanine in the excreta of specimens of *Androctonus australis* and *A. amoreuxi,* but failed to detect either adenine or hypoxanthine. In India, Kanungo, Bohidar and Patnaik (1962) reported adenine, hypoxanthine, and uric acid, but not guanine or xanthine in excreta of *Palamnaeus bengalensis.* An extensive study by Rao and Gopalakrishnareddy (1962) showed that guanine was the most abundant purine excreted by the scorpions, *Heterometrus fulvipes, Lycas tricarinatus, Buthus tamulus,* and the uropygid, *Thelyphonus sepiaris.* Uric acid and hypoxanthine were of lesser importance. From these accounts scorpions seemingly differ both qualitatively and quantitatively in the purines excreted. Because arachnids consume considerable amounts of uric acid from their insect prey, the role of diet as an influential factor affecting purine excretion in scorpions and uropygids has been investigated. An evaluation of the malpighian tubules in purine excretion in a centipede (Chilopoda) and a scorpion also is presented.

Methods

Excreta of the following arthropods was analyzed.

Class Arachnida:

Order Scorpiones:	Buthidae	*Centruroides marginaritatus*
	Buthidae	*Centruroides vittatus*
	Scorpionidae	*Heterometrus* sp.
	Vejovidae	*Vejovis* sp.
	Vejovidae	*Vejovis mexicanus*
Order Urogpygi:		*Mastigoproctus giganteus*

Class Chilopoda:

Order Scolopendromorpha: *Scolopendra heros*

With the exception of *C. marginaritatus* from Florida, U. S. A. and *Heterometrus* sp. from Bangkok, Thailand, all species were from Central and West Texas, U. S. A. The two undescribed species of scorpions are being described by Dr. H. Stahnke, Arizona State University. The local arthropods were maintained in the laboratory for many weeks and fed crickets (*Gryllus assimilis*). Faeces were collected for several weeks following feeding to determine if uric acid might be exogenous, and several milligrams of excreta were dissolved by heating in 0.1 N NaOH for a few minutes. The spectrophotometric method of Vischer and Chargaff (1948) and the modified phosphotungstic acid method (Eichhorn, Zelmanowski, Lewis and Fanias (1961) were used to quantitatively determine guanine and uric acid. Qualitative tests for guanine were according to Hawk, Oser and Summerson (1954). Ascending paper chromatography was used to qualitatively test for other purines. Chromatograms were developed with the mercury-diphenylcarbazone reagent of Dikstein, Bergman and Chaimovitz (1956). The solvents were as follows: (1) 95% ethanol: aqueous 0.4 N NaOH (3:1); (2) 99% isopropanol: aqueous 0.4 N NaOH (3:1); (3) isoamyl alcohol and 5% KH_2PO_4 (Carter, 1950). Total nitrogen was estimated by the microkjeldahl method (Hawk *et al.*, 1954).

TABLE I

Analysis of excreta purines of seven terrestrial arthropods

Species	% of Total excreta nitrogen	% Guanine-N of total nitrogen	% Uric acid-N of total nitrogen
Centruroides vittatus (14)*	39.1 ± 6.3	44.6 ± 14.8	0.12 ± 0.27
C. marginaritatus (1)*	—	40.0	1.8
Vejovis mexicanus (3)*	39.4 ± 3.1	91.0 ± 1.5	ND***
Vejovis sp. A** (12)*	31.9 ± 11.0	81.0 ± 20.0	0.30 (1); ND (11)
Heterometrus sp. B** (5)*	41.0 ± 10.0	63.2 ± 10.1	ND
Mastigoproctus giganteus (13)	36.7 ± 12.4	74.2 ± 27.8	1.1 (1); ND (12)
Scolopendra heros (3)	8.4 ± 0.4	ND	12.5 ± 6.0

* Number of analyses.

** Undescribed species of scorpions currently being described by Dr. H. Stahnke, Arizona State University. (A) from Jeff Davis Co., Texas; (B) from Bangkok, Thailand.

*** Not detectable.

Results and Discussion

Analyses of excreta are presented in Table I. All of the arachnids excrete primarily guanine and some species inconsistently void uric acid. No other purines were found by chromatographic and spectrophotometric techniques which would have detected quantities equivalent to 5% TN. Uric acid analyses of *C. vittatus* excreta collected for a two week period following feeding are extremely variable (Table II). Most excretory samples did not contain detectable levels of

TABLE II

Percentage uric acid excreted by Centruroides vittatus *following feeding*

Number of scorpions	Time in days since feeding			
	4	8	12	16
1	0.25	1.50	4.90	0.00
1	0.20	0.50	0.00	0.00
1	0.10	1.20	2.60	0.00
2	0.00	0.00	—	—
3	0.00	0.70 ± 0.27*	—	—
6	0.00	0.00	0.00	0.00

* Mean ± Standard Deviation.

uric acid. Those individuals that do excrete some uric acid do so in quantities ranging from trace amounts to 4.9%.

Uric acid was rarely found in excreta of starved animals and was detected only once in the excreta of a vejovid scorpion and the vinegarroon, *Mastigoproctus giganteus.* In these arachnids, the role of uric acid in nitrogen excretion seems of little consequence.

Other workers have reported higher levels of uric acid in scorpion excreta. For instance, specimens of the Indian scorpions, *Heterometrus fulvipes, Lycas tricarinatus,* and *Buthus tamulus* and the African scorpion, *Buthus quinqestriatus,* excreted 8–12% and 3% of their total nitrogen as uric acid. However, all of the values of Rao and Gopalakrishnareddy (1962) on the Indian scorpions apparently are based on only one sample, except those values for *H. fulvipes* excreta where at least two samples were reported. Because of the small sample size an attempt to evaluate the physiological importance of uric acid excretion in these species is impractical. In contrast the uric acid values reported by Haggag and Fouad (1965) for *B. quinquestriatus* excreta were based on 15 specimens and therefore suggest that this buthid scorpion consistently excretes uric acid. In the present study, only the buthid scorpion excreta frequently contained uric acid, and even then usually in low concentrations. That there are both intra- and interspecific differences in the patterns of uric acid excretion even when diet is controlled suggests that the buthid scorpions truly differ from the other species in aspects of uric acid metabolism.

With the exception of *C. marginaritatus* and *C. vittatus,* guanine always accounted for more than 63% of the fecal nitrogen. Adenine and hypoxanthine were never detected. Spectral curves of excreta were almost superimposable on a standard guanine curve of similar concentration—thus illustrating the absence of interfering compounds (*e.g.,* other purines). Occasional exceptions were noted in the spectral curves of *C. vittatus* excreta where peak absorption occurred at 280 instead of 273 mu. In these samples guanine was not quantitatively determined. Low concentrations of xanthine might account for the shift of the spectral curve, even though none was detected by paper chromatographic techniques. Similar guanine concentrations have been reported in other scorpions (Rao and Gopalakrishnareddy, 1962; Haggag and Fouad, 1965), whereas high levels of hypoxanthine were never found in the excreta of the buthid scorpion, *C. vittatus,* as it was in *Buthus tumulus* (Rao and Gopalakrishnareddy, 1962); nor was adenine detected as it was in *Palamnaeus bengalensis* (=*Heterometrus bengalensis*) (Kanungo *et al.*, 1962). In the excreta of the Indian uropygid, *T. sepiaris,* guanine accounted for 69.5% of the total nitrogen, while corresponding values for the American species, *M. giganteus* were 74.2%. Seemingly guanine is also the primary nitrogenous waste of uropygids. Uric acid was found in detectable levels in only one of twelve samples of excreta of *M. giganteus.*

Scorpions seemingly differ in patterns of purine excretion. Whether these are true differences in scorpion nitrogen metabolism or just variations resulting from dietary purines is not known. For uric acid the latter possibility seems most feasible because arachnids feed principally on uricotelic insects. This view also is supported by the many uric acid analyses of the current study which show no consistent pattern in uric acid excretion, whereas guanine is consistently excreted by all arachnids. Working with 34 species of spiders that represent 17 families, Anderson (1966) found that guanine was the only purine excreted in detectable amounts, whereas earlier workers had reported uric acid also from spider excreta (Kanungo *et al.,* 1962; Haggag and Fouad, 1965). Since only small percentages of uric acid have been reported, dietary uric acid might account for these conflicting reports. Of the total excreta nitrogen, 0.26% was attributed to uric acid in the spider, *Lycosa urbana* (Haggag and Fouad, 1965). An opposite situation was reported in piscivorous sea birds. Guanine in the bird excreta was found to be exogenous because it was only detected when the birds were fed fish (Keilin, 1958).

To determine the importance of the malpighian tubules in guanine excretion, samples from both the central tube of the midgut and hindgut of the large Thai scorpion, *Heterometrus* sp., were analyzed. By employing spectrophotometric techniques and qualitative guanine tests (Hawk *et al.*, 1954) the white material from both regions of the gut tube and from the mesenteron were identified as guanine. Such deposits are scattered throughout the mesenteron and in the diverticula leading into the central tube of the midgut. Synthesis of guanine apparently occurs in the mesenteron where excretion occurs directly into the midgut tube via the diverticula. Problems associated with transporting such an insoluble compound from the site of synthesis to that of excretion are avoided. That the transparent malpighian tubules were never noted to contain guanine crystals suggests that they are of lesser importance in guanine excretion than the mesenteron. Similar findings have been reported in some spiders (Barnes, 1968). In a few insects the gut is more important than the malpighian tubules in uric acid excretion (Wigglesworth, 1953).

Though measurable levels of uric acid have been reported in the coxal glands of the scorpions, *H. fulvipes* and *B. tamulus,* the hindgut contents contain much higher concentrations of uric acid which are of the same magnitude as those in the excreta (Rao and Gopalakrishnareddy, 1962). The coxal glands, therefore, probably are of little consequence in nitrogen excretion when compared with the mesenteron and malpighian tubules. It is possible that some of the variation in uric acid excretion might be due to irregular emptying of the coxal glands, but most of the uric acid reported from excreta comes from the gut as indicated above, and may actually be exogenous in origin.

Ample amounts of uric acid nitrogen (12%-TN) are voided with the faeces in the centipede, *Scolopendra heros.* Because centipedes consume both hard and soft body parts, the uric acid contribution to the total fecal nitrogen is low. Chitin and other undigestible nitrogenous compounds contribute more to the total nitrogen values than in arachnids which feed mainly on the easily digested soft body parts. Centipede excreta contain 8.4%-N dry wt. while arachnid excreta contain about 36%-N dry wt.

As in other mandibulates the centipede malpighian tubules are implicated as sites for uric acid excretion. Uric acid analyses of the midgut contents from just anterior to the junction of the malpighian tubules and the hindgut show uric acid levels of 0.9%, while a pool of the hindgut contents and malpighian tubules has 17% uric acid. Obviously, the centipede uric acid is not dietary and probably is excreted into the hindgut via the malpighian tubules.

From an evolutionary standpoint it is interesting that among the terrestrial arthropods the arachnids excrete guanine as their principle nitrogenous waste product whereas chilopods and insects excrete uric acid. Advantages to guanine production and excretion might be that it allows for more efficient water conservation. Guanine contains one more nitrogen atom than uric acid and is less soluble in water. Both of these properties would be of value to animals dwelling in hot, dry climates where water retention would be essential. Urea, which is highly water soluble, has not been reported in arachnids (Anderson, 1966; Rao and Gopalakrishnareddy, 1962).

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Summary

1. Effects of diet on uric acid excretion of three scorpions, *Centruroides vittatus, Vejovis mexicanus* and *Vejovis* sp., and the vinegarroon, *Mastigoproctus giganteus,* have been investigated. Uric acid probably is not a normal end product of nitrogen catabolism, except possibly in *C. vittatus.* Yet even here the pattern is inconsistent. Quite likely the uric acid in arachnid excreta is exogenous in origin.

2. The most important nitrogenous waste in these arachnids was guanine. In contrast to other reports on African and Asian scorpions, uric acid and hypoxanthine are of little consequence in the North American species.

3. In the large Thai scorpion, *Heterometrus* sp., the malpighian tubules are of lesser importance in guanine excretion than the mesenteron. Apparently guanine is synthesized in the mesenteron and passes via the diverticula into the gut canal rather than being excreted by the malpighian tubules.

4. Considerable quantities of uric acid are excreted in the centipede, *Scolopendra heros,* probably via the malpighian tubules.

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